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Lab Resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line from urinary cells of a healthy donor using integration free Sendai virus technology

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ABSTRACT

We have generated a human induced pluripotent stem cell (iPSC) line derived from urinary cells of a 28 year old healthy female donor. The cells were reprogrammed using a non-integrating viral vector and have shown full differentiation potential. Together with the iPSC line, the donor provided blood cells for the study of immunological effects of the iPSC line and its derivatives in autologous and allogeneic settings. The line is available and registered in the human pluripotent stem cell registry as BCRTi005-A.

Resource table

Name of stem cell construct	BCRTi005-A
Institution	Charité – Universitätsmedizin Berlin, Berlin Brandenburg Center for Regenerative Therapies (BCRT)
Person who created resource	Bella Rossbach
Contact person and email	Andreas Kurtz (Andreas.Kurtz@charite.de)
Date archived/stock date	February, 2015
Origin	Cells isolated from human urine
Type of resource	Human induced pluripotent stem cells (hiPSCs) derived from a healthy female donor
Sub-type	hiPSC line
Key transcription factors	OCT4, SOX2, MYC, KLF4
Authentication	STR analysis/fingerprinting
Link to related literature (direct URL links and full references)	
Information in public databases	http://hpscreg.eu

(See Figs. 1–4.)

Material and methods

Ethical statement

This work was approved by the Ethics Commission of the Charité – Universitätsmedizin Berlin (EA4/110/10).

Urinary cell isolation and expansion

Urine was obtained from a female healthy Caucasian donor of 28 years of age. Urinary cells were isolated according to the protocol described by Zhou et al. (2012). After centrifugation and washing with PBS (Gibco) the obtained cell pellet was resuspended in primary-medium containing DMEM high glucose (GIBCO) and HAM's F12 (Biochrom), which were mixed in a 1:1 ratio and supplemented with 2,5 µg/ml amphotericin B (Biochrom), 10% (v/v) fetal bovine serum (FBS, Biochrom), penicillin/streptomycin (P/S, 100 U/ml/100 µg/ml, Biochrom) and the renal growth media (REGM) SingleQuot kit (Lonza) and finally plated on 0,1% w/t gelatin coated (Milipore) plates. Primary-medium was added for the following three days and afterwards successively changed to proliferation-medium which was a 1:1 mixture of DMEM high glucose and the REBM basal medium supplemented with 5% (v/v) FBS, 1,25 mM GlutaMax™ , 0,5% (v/v) non-essential amino acids (NEAA, Gibco), P/S (50 U/ml, 50 µg/ml), 2,5 ng/ml basic fibroblast growth factor (bFGF), 2,5 ng/ml platelet derived growth factor (PDGF-AB), 2,5 ng/ml human epidermal

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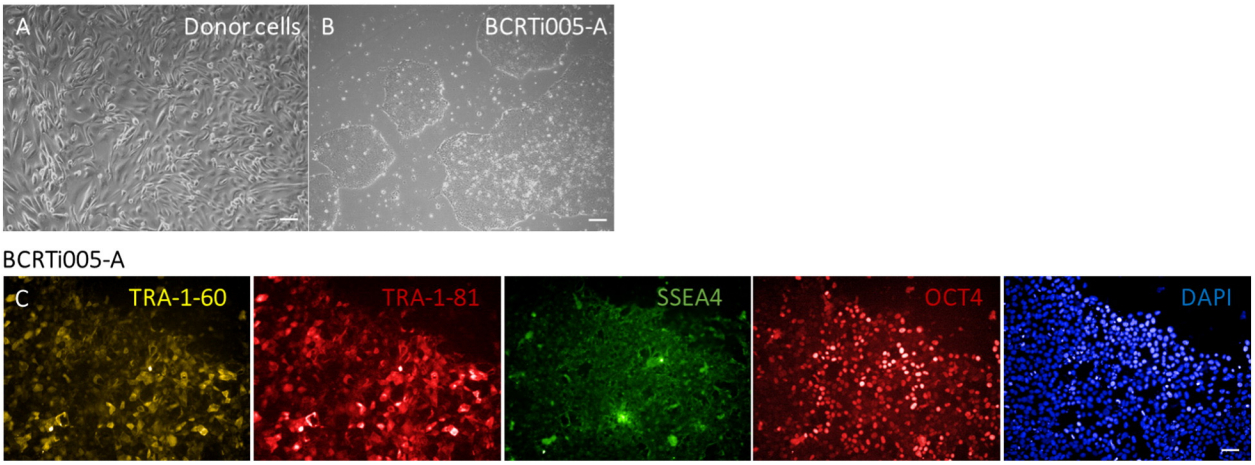

Fig. 1. Morphology of primary urinary cells, reprogrammed hiPSCs and pluripotency marker expression. (A) Phase contrast images of primary urinary cells and (B) generated hiPSCs. (C). hiPSC lines were stained for the pluripotency markers TRA-1-60, TRA-1-81, SSEA-4, OCT4 and DAPI for staining of nuclei. Scale bars: A, B and: 100 μm, C: 50 μm.

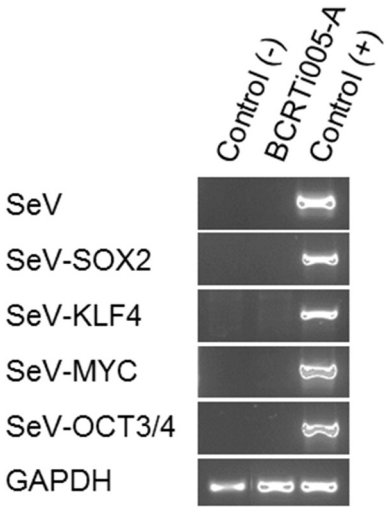

Fig. 2. Analysis of Sendai viral transgene expression in generated hiPSC lines. RT-PCR of reprogrammed hiPSC lines was performed for the expression of Sendai virus (SeV), SeV-SOX2, SeV-KLF4, SeV-MYC and SeV-OCT3/4.

growth factor (hEGF, all Peprotech) and the REGM SingleQuot kit. First colonies appeared on day 5. After reaching about 90% confluency, cells were passaged using trypsin (Biochrom) and re-plated on gelatin coated dishes in a ratio of 1:10. Cells were frozen in FBS containing 10% dimethylsulfoxid (DMSO).

Reprogramming and hiPSC maintenance

Urinary cells were thawed and cultivated in RPMI (Gibco) medium supplemented with 10% FBS and 1% P/S on gelatin coated dishes until reaching confluence of around 90% again. The Sendai virus (SeV) based non-integration CytoTune™ iPS Reprogramming Kit (Life Technologies) (Fusaki et al., 2009) was used according to manufacturer's instructions. First colonies appeared after 10 days. 5 single colonies were picked and cultivated under feeder free conditions on Geltrex (Life Technologies) in TeSR-E8 medium (Stemcell Technologies) supplemented with 1% P/S. The hiPSCs were passaged every 5–7 days in a ratio of 1:6. At passage 14, colonies were tested for virus loss and pluripotency marker expression and one of the 5 colonies was kept in culture for further analysis. Frozen stocks of the other colonies were kept too.

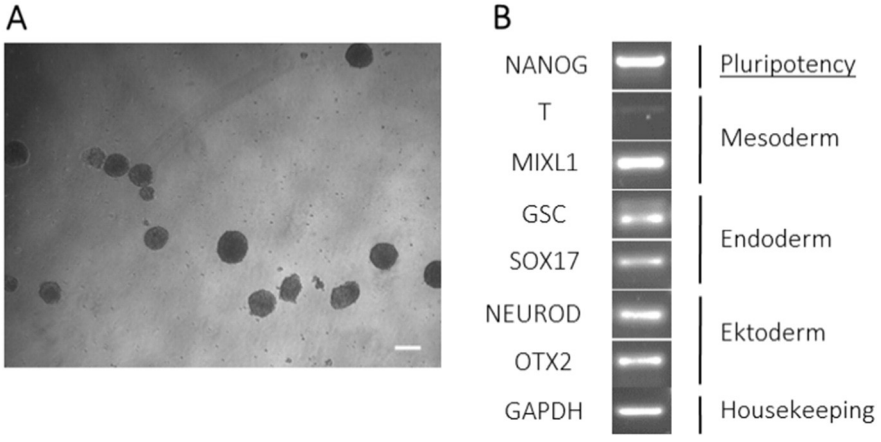

Fig. 3. Spontaneous in vitro differentiation of hiPSCs into cells of the three germ layers. (A) EB formation and (B) RT-PCR for the markers NANOG, Brachyury (T), Mix Paired-like Homeobox (MIXL1), Goosecoid (GSC), SRY (Sex Determining Region Y)-Box 17 (Sox17), NeuroD, Orthodenticle homeobox 2 (OTX2) and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Scale bar: 100 μm.

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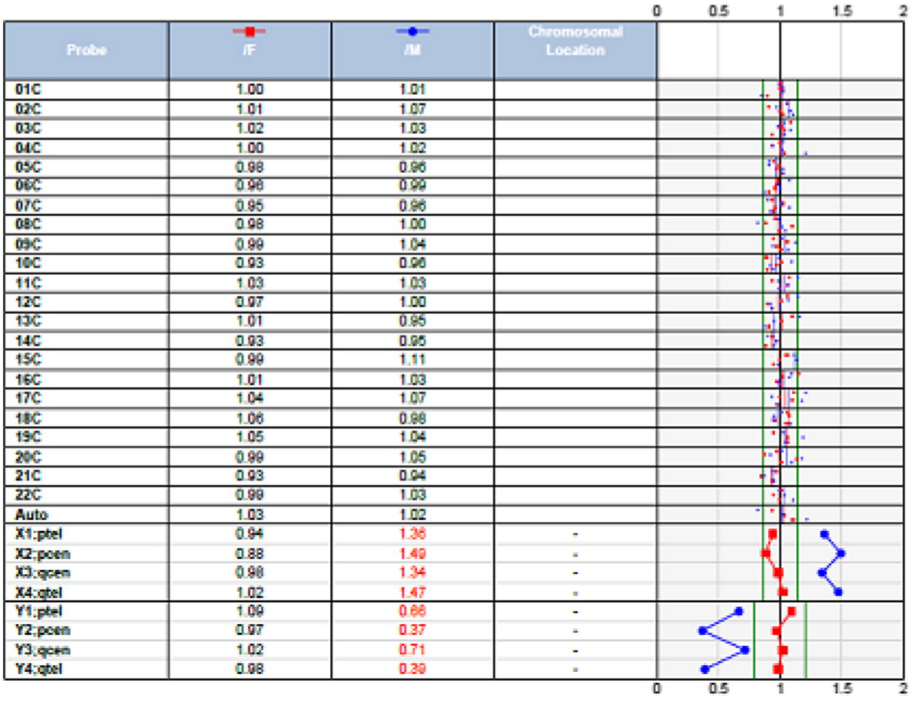

Probe	/F	/M	Chromosomal Location
01C	1.00	1.01	
02C	1.01	1.07	
03C	1.02	1.03	
04C	1.00	1.02	
05C	0.98	0.96	
06C	0.98	0.99	
07C	0.95	0.96	
08C	0.98	1.00	
09C	0.99	1.04	
10C	0.93	0.96	
11C	1.03	1.03	
12C	0.97	1.00	
13C	1.01	0.95	
14C	0.93	0.95	
15C	0.99	1.11	
16C	1.01	1.03	
17C	1.04	1.07	
18C	1.06	0.98	
19C	1.05	1.04	
20C	0.99	1.05	
21C	0.93	0.94	
22C	0.99	1.03	
Auto	1.03	1.02	
X1:ptel	0.94	1.36	-
X2:pcen	0.88	1.49	-
X3:qcen	0.98	1.34	-
X4:qtel	1.02	1.47	-
Y1:ptel	1.09	0.68	-
Y2:pcen	0.97	0.37	-
Y3:qcen	1.02	0.71	-
Y4:qtel	0.98	0.39	-

Fig. 4. Karyotyping of hiPSC lines. Karyogram was determined using the KaryoLite™ BoBs™ Kit. Female and male DNA with normal karyotypes were used as reference and chromosomal signals were compared with the tested hiPSC DNA (red: against female, blue: against male DNA). No aneuploidies were detected for the hiPSC line BCRTi005-A.

Pluripotency marker staining of hiPSCs

hiPSCs were fixed (BD Cytofix) and permeabilized using Perm/Wash buffer (BD) and stained with antibodies against the pluripotency markers TRA-1-60 (Novous Biologicals), TRA-1-81 (Novus Biologicals), OCT-4 PerCP-Cy5.5 (BD) and SSEA-4 FITC (R&D). Subsequently, cell nuclei were labeled with 4′,6-diamidino-2-phenylindole (DAPI, Invitrogen). The final analysis was performed using the High Content Screening Imaging System Operetta (Perkin Elmer).

RNA isolation and cDNA synthesis

Total RNA was extracted (Qiagen RNA Mini Kit) and transcribed into cDNA using the SuperScript® III Transcriptase Kit (Life Technologies).

Detection of SeV genome and transgenes

After 14 passages, hiPSC lines were tested for SeV residues. PCR was performed using primers and instructions (Table 1) as recommended by the manufacturer. As positive control RNA was used from the reprogramming leftovers. Negative control RNA was obtained from the hiPSC line WISCi004-A (IMR90, WiCell), which was lentivirally reprogrammed from IRM90.4 fibroblasts.

Embryoid body (EB) formation and germ layer differentiation

To test the capacity of the reprogrammed hiPSC line to spontaneously differentiate into cells of all three germ layers, hiPSCs were harvested using accutase (Gibco) and seeded in TeSR-E8 supplemented with 10 μm Rock inhibitor (StemCell Technologies) into AggreWell plates 800 (StemCell Technologies). EBs formed within 24 h and were further cultivated on ultra-low attachment plates (Corning) in KO-DMEM (Invitrogen) supplemented with 20% Knockout SerumReplacer (Invitrogen), 1,25 mM GlutaMax™, 1% (v/v) NEAA, 0,2% (v/v) beta-Mercaptoethanol (Gibco) and P/S (100 U/ml/100 μg/ml) for 10 days. Medium was changed every 48 h.

EBs were harvested and isolated RNA was transcribed into cDNA. PCR was performed to analyze the expression of germ layer specific genes (Table 2). The following PCR program was used: 96 °C for

Table 1
Primer sequences for Sendai viral test.

Target		Primer sequence
SeV	Forward	AAGGTCCCGGTCAAGAAACAG
	Reverse	CTTCTGCGTCACACCATTGC
SOX2	Forward	TAAGGTGGATCTTCAGGTAGC
	Reverse	CATCTCATTGGTGAGCTCCCT
MYC	Forward	CTGTTCCCCTCTCTCTGAAGA
	Reverse	GGCAGAAAAGATGTGTTCCTCC
OCT3/4	Forward	AACGCGGAGAAGTGGAACAAG
	Reverse	CTGTCCGAGTCCAAATCGC
KLF4	Forward	GTGGACCGCACGGAATTTG
	Reverse	GGAGATTCACACCGGAGTCA

Table 2
Primer sequences for pluripotency and germ layer specification.

Target		Primer sequence
NANOG	Forward	AAGGTCCCGGTCAAGAAACAG
	Reverse	CTTCTGCGTCACACCATTGC
Brachyuri (T)	Forward	TAAGGTGGATCTTCAGGTAGC
	Reverse	CATCTCATTGGTGAGCTCCCT
MIXL1	Forward	CTGTTCCCCTCTCTCTGAAGA
	Reverse	GGCAGAAAAGATGTGTTCCTCC
GSC	Forward	AACGCGGAGAAGTGGAACAAG
	Reverse	CTGTCCGAGTCCAAATCGC
SOX17	Forward	GTGGACCGCACGGAATTTG
	Reverse	GGAGATTCACACCGGAGTCA
NEUROD1	Forward	GCCCCAGGGTTATGAGACTATCACT
	Reverse	CCGACAGAGCCCAGATGTAGTTCTT
OTX2	Forward	TGTAGAAGCTATTTTTGTGGGTGA
	Reverse	GAGCATCGTTCCATCTAACTTTTT
GAPDH	Forward	TTGCCATCAATGACCCCTTCA
	Reverse	CGCCCCACTTGATTTTGGA

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5 min, 35 cycles of 96 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s. Final elongation was done at 72 °C for 7 min.

Karyogram

For the detection of chromosomal aberrations, the KaryoLite™ BoBs™ Kit was used supplied by Perkin Elmer (Lund et al., 2012). Genomic DNA was processed according to the manufacturer's instructions. Female and male controls were used in parallel. The fluorescent signal was detected using the Bio-Plex 200 system (BioRad) and finally analyzed using the Perkin Elmer software BoBsoft™.

Fingerprinting

Short Tandem Repeat (STR) analysis was performed to identify the purity of these cell lines. DNA was extracted (Qiagen) from the hiPSCs and the corresponding primary cells. The genome was analyzed for 10 different loci out of the Combined DNA Index System (CODIS): D16S539, D7S820, CSF1PO, D5S818, TPOX, D13S317, vWA, HUMTH01, DXS101 and DYS393.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2016.09.002.

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